IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket: TOVEY5A

In re Application of:) Conf. No.: 1014
)
Michael G. TOVEY et al.) Art Unit: 1636
)
Appln. No.: 10/677,777) Examiner: L. Mitchell
)
Filed: October 3, 2003) Washington, D.C.
)
For: GENE REPORTER ASSAY, AND)
CELLS FOR DETERMINING THE)
PRESENCE AND/OR THE …)

DECLARATION UNDER 37 CFR §1.132

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Arlington, Virginia 22202

Sir:

I, Allen C. Yun, hereby declare and state as follows:

I am an agent registered to practice before the U.S. Patent and Trademark Office (Reg. No. 37,971). I am employed by the law firm of Browdy and Neimark, PLLC.

I was assigned the task of preparing the patent application that was eventually filed on October 4, 2002, as U.S. provisional application 60/415,818 (given the Browdy and Neimark docket no. TOVEY5) and of which the present application claims the benefit of priority.

The invention claimed in the present application was disclosed to me and to Roger L. Browdy, who is a Member (equivalent to a partner) of Browdy and Neimark, PLLC, at a meeting with Dr. Michael G. Tovey, a co-inventor, prior to September 20, 2002. A memorandum summarizing this meeting with Michael Tovey prior to September 20, 2002, was dictated by Mr. Browdy at the time of the meeting. A copy of this contemporaneously prepared memorandum is submitted herewith as Exhibit A. The date of this memorandum is excised but this date is also prior to September 20, 2002. Also excised from this memorandum are meeting notes on discussions and topics not immediately directed to the invention disclosure.

From the discussion at the meeting with Michael Tovey, as evidenced by the attached memorandum, I understood that conception of the invention was completely described to me prior to September 20, 2007.

Also submitted herewith as Exhibit B is a copy of a computer printout of my time spent (time sheet) in the preparation of the provisional application filed October 4, 2002, obtained from the computer program I used during that period to keep track of my time.

It was my habit during this period to write down each day the time I spent on a project/application/case and then to input this information within one or two business days into the computer along with brief details of the type of work that was done during that time.

The computer printout of my time sheet (Exhibit B) shows that I diligently worked on the preparation of the provisional application at least part of every business day from a date immediately prior to September 20, 2002, to the October 4, 2002, filing of U.S. provisional application 60/415,818. The column of numbers presented in Exhibit B immediately to the left of the brief description of the type of work done represents the time spent each day in hours. Thus, for example, according to the computer printout of my timesheet, I had spent 8.10 hours on September 19, 2002, drafting claims and the application.

The evidence presented hereby establishes that the invention described and claimed in the above-identified patent application was conceived in the United States prior to September 20, 2002, and that I exercised reasonable diligence in drafting the patent application that serves as constructive reduction to practice of this invention from September 19, 2002, through the date of filing of provisional application 60/415,818 on October 4, 2002. Accordingly, a date of invention prior to September 20, 2002, has now been established.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

November 8, 2007
Date

/ACY/
Allen C. Yun

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EXHIBIT A

MEMORANDUM

To: File

From: Roger L. Browdy

Date:

Subj: Meeting with Michael Tovey

I. Gene Reporter Assay

The first invention discussed relates to a Gene Reporter Assay. Essentially, there are many assays that may rely on *in vivo* activity in a living cell line. One example is a cell line having an Interferon Sensitive Response Element (ISRE) connected to a luciferase gene, or another reporter gene, so that when the cell line is subjected to the presence of interferon the endogenous interferon cell surface receptors produce a signal that activates the ISRE, which then causes transcription of the luciferase gene. Thus, the activity of luciferase in creating light can be measured and is related to the amount of interferon which is present in the sample, and which is directly proportional to the amount of interferon over a particular range.

With such types of cell lines which can be used in such assays, there is a problem in commercializing them in that once the cell line is shipped for use in a single assay, the laboratory can grow the cells and use their own stock of cells for future assays. Thus, it is an object of the present invention to find a way to essentially make the commercial cell line a "one-use" cell line so that every time a laboratory wants to run the assay, it must purchase the cell line from the supplier. Another problem is that living cell lines have no shelf life. It is also an object of the invention to prepare such a one-time use cell line that maybe stored for a period of time to allow for shelf life and shipping.

It has been discovered that these objects can be achieved by treating the cell line in such a manner that it can no longer replicate but it retains its signal transduction activity. One way to do this is by means by γ irradiation. It is known that irradiation at a particular dose will cause the cell to lose its signal transduction activity. Irradiation at a somewhat lower dose will cause replication of this cell line to be inhibited. It has now been discovered that it is possible to determine a dose which inhibits replication but still allows the cell to be used for its signal transduction assay. For example, irradiation at 9 Gray allows the cells to remain viable for 14 days, after which replication is inhibited and the cells undergo apoptosis. However, during those 14 days the signal transduction assay works as well as in a non-irradiated control. This provides a product with a 14-day shelf life, but which becomes inactive after this amount of time so that it cannot be maintained and reproduced by the laboratory.

A second way to achieve the objects of the invention is by means of use of an antimitotic agent. An antimitotic agent will kill the cells when they begin to replicate. Thus, cells which have been pretreated with an antimitotic agent will have a shelf life of about 24 hours during which the signal transduction assay can be conducted and after which the cells will die. Obviously, 24 hours is insufficient. In order to extend the shelf life, the pretreated cells may be immediately frozen, in which state they will have a much longer shelf life, depending upon the manner of freezing and thawing. Once thawed, however, they must be used within 24 hours, after which they will die.

It should be understood that conventional wisdom is that cryopreservation of cell lines requires a special freezing and thawing process where the cells are frozen at a rate of 1° per minute until it reaches -190°C, where it may be stored indefinitely, after which it must be thawed very rapidly. Often, DMSO or another cryopreservative is also used in order to help protect the cells. If the cells are pretreated with antimitotic agent, then they can be frozen with this cumbersome cryopreservation technique for an indefinite period and then be used for the purpose of the signal transduction assay for 24 hours after being thawed. However, this is not a commercially viable technique as it would greatly increase the manufacturing cost of commercialization.

As most labs do not have storage facilities at -190°C, it would be useful to allow freezing at -20°C. However, it is known that cells die when frozen at -20°C. In the course of the experimentation leading to the present invention, it was unexpectedly discovered that DMSO will protect the cells even when frozen at -20°C without any special freezing or thawing techniques. It is known that cryopreservative compounds such as glycerol will protect cells at -20°C. However, compounds such as glycerol prevent protein ligands from interacting with surface receptors. DMSO does not have this disadvantage. It is a discovery of the present invention that DMSO will protect cells frozen at -20°C without any special freezing or thawing techniques being required, without adversely affecting their sensitivity to IFN. Thus, it is another surprising and inventive aspect of the present invention that after pre-treating with an antimitotic agent, the cell line may achieve a long shelf life even at standard freezer temperatures if further pretreated with DMSO and that once thawed such cells will remain active for signal transduction assays for approximately 24 hours, until killed by the antimitotic agent. Any agent which kills cells during the process of replication can be used for this purpose as it would be expected that the cells will remain biologically active until during the quiescent period and until they start to replicate.

While the two methods discussed above are operable in the course of the present invention, other methods of accomplishing the objectives failed. For example, it is known that by treating the cells with a fixing agent such as paraformaldehyde or glutaraldehyde, the cells will die but they will remain fixed such that the surface proteins are not denatured. Thus, it was thought that perhaps the enzymatic activity of the signal transduction activity may remain even after the cells have been fixed so that they can no longer replicate. This was found not to be the case at any of the tested dosages of fixing agent. Thus, all of the fixing agents tested at all of the doses tested completely abrogated out the ability of the cells to respond to interferon.

Another method that failed was to use an antimitotic or pro-apoptotic agent, such as phenylarsine oxide or aurintricarboxylic acid, at doses lower than the effective dose to see if an extended shelf life could be obtained during which the signal transduction assay could still be conducted before the cells died. No such dose could be found. Thus, it is surprising that an effective γ irradiation dose for this purpose could be found.

While the above discussion is a generalized discussion for being able to commercialize any cell line engineered for signal transduction assays, such as those described in the Harpel et al. patents 5,436,128 and 5,401,629, there are also specific advantages to the interferon assaying cell lines that are used in the present examples. Such cell lines have many important utilities. For example, they can be used as a surrogate marker for viral infection. It is known that circulating interferon is indicative of a virus infection but circulating interferon does not appear in a person who has a bacterial infection. Thus, if a person is suspected of having an infection and one wants to determine whether the infection is viral or bacterial, this assay would be extremely useful. In HIV patients, it is known that the circulating interferon level becomes detectable towards the end of the asymptomatic period. Thus, interferon monitoring is indicative of disease progression.

Secondly, it can be used as a surrogate marker for detecting viral infections caused by bioterrorism in a susceptible population. If random blood samples are taken in a susceptible population, this simple gene reporter assay allows a relatively fast and inexpensive determination of ubiquitous viral infection which would be an indication of a possible bioterrorism attack.

Furthermore, rat cell lines, bovine cell lines, mosquito cell lines, etc., can be created which have the ISRE reporter gene in order to determine if animals are infected with a virus, such as to detect bubonic plague in rats, West Nile virus in mosquitos, hoof and mouth disease in cattle, etc.

A third utility is to monitor interferon therapy to see how much interferon is actually entering the bloodstream and to determine patient compliance.

Finally, it can be used to detect autoimmune diseases, most of which are characterized by the presence of interferon. Often these diseases have stages of exacerbation and remission with an increase in circulating interferon prior to an exacerbation phase. Thus, this assay can be used to detect when a patient might be ready to enter an exacerbation stage.

Prior art techniques of interferon assay include bioassay of viral activity. This takes a minimum of five days and has a relatively high threshold of interferon activity. Another possible way is by means of antibody based ELISA assays. However, this technique has a threshold perhaps a thousand-fold higher than the present test, and is specific for one specific kind of interferon only.

The present assay recognizes any interferon which binds to IFN receptor. It is possible, however, to determine which kind of interferon is being detected by using a parallel antibody treatment. Thus, an antibody for a specific type of interferon will prevent that interferon from causing signal transduction. If the signal disappears in the presence of antibody, then it is known that the particular interferon being detected is that to which the antibody is specific. The activity curve for different types of interferon differ in any given reporter cell line. Activity curves can be generated for each type of interferon. Using such standard curves, one can accurately quantitate the interferon present in the serum sample once it is known what type of interferon is being detected.

Besides luciferase any type of reporter gene can be used. A particularly desirable one is EGFP, enhanced green fluorescent protein. This is preferred as the fluorescence can be measured directly without lysing the cells, subjecting them to luciferin, etc.

EXHIBIT B

TOVEY	-USA	09/19/02	8.10	DRAFT CLAIMS AND APPL
TOVEY5	-USA	09/20/02	5.80	DRAFT CLAIMS, APPL
TOVEY5	-USA	09/21/02	0.75	DRAFT APPL
TOVEY5	-USA	09/22/02	4.00	DRAFT APPL
TOVEY5	-USA	09/23/02	4.50	MEETING W/M.TOVEY
TOVEY5	-USA	09/24/02	3.90	DRAFT CLAIMS, APPL; EMAIL CORRESP
TOVEY5	-USA	09/25/02	6.45	DRAFTING APPL
TOVEY5	-USA	09/26/02	8.50	DRAFT APPL
TOVEY5	-USA	09/27/02	8.25	DRAFT APPL, EMAIL
TOVEY5	-USA	09/30/02	0.70	RESEARCH LUCLIT PLUS
TOVEY5	-USA	10/01/02	0.20	EMAIL
TOVEY5	-USA	10/02/02	0.10	EMAIL
TOVEY5	-USA	10/03/02	4.90	TELECONF W/M.TOVEY, REVISIONS
TOVEY5	-USA	10/04/02	3.30	REVISIONS AND PROOFING, FIGS, MISC